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Temperature-dependent resolution of conflict over rank within a size-based dominance hierarchy

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Abstract

In social groups, hierarchies are the fundamental organizational unit and integral to the structure of social groups. For many social fishes, rank is determined by body size and conflict over rank is resolved via aggressive threats from dominants and growth restraint by subordinates. However, this balance may be offset by an alteration of abiotic factors, such as elevated temperature expected from climate change, which could thereby disrupt the usual mechanisms of conflict resolution. Here, we determined the effect of elevated temperature on hierarchy structure, stability, and conflict resolution in the Eastern mosquitofish, Gambusia holbrooki. Body size was significantly related to dominance rank, and aggression was more commonly directed toward subordinates and was heightened between individuals of adjacent rank, demonstrating that conflict over rank occurs in size-based hierarchies. Temperature did not affect overall levels or directionality/adjacency of aggression but substantially altered subordinate growth patterns. In only the high-temperature groups, growth rates of subordinates decreased as the size ratio between themselves and their immediate dominant approached 1.0, whereas growth rates of dominants were unaffected. This unique finding suggests that only under high temperatures, subordinates may adopt growth regulation to resolve conflict, when the costs of conflict with dominants are greater. This provides the first causal link between abiotic stressors and changes to hierarchical structure and functioning, providing a springboard for further research into implications of temperature-dependent subordinate growth alteration at higher levels of ecological organization.

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Temperature-dependent resolution of conflict over rank within a size-based dominance hierarchy

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ABSTRACT

In social groups, hierarchies are the fundamental organisational unit and integral to the structure of social groups. For many social fishes, rank is determined by body size and conflict over rank is resolved via aggressive threats from dominants and growth restraint by subordinates. However, this balance may be offset by an alteration of abiotic factors, such as elevated temperature expected from climate change, which could thereby disrupt the usual mechanisms of conflict resolution. Here we determined the effect of elevated temperature on hierarchy structure, stability and conflict resolution in the Eastern mosquitofish, *Gambusia holbrooki*. Body size was significantly related to dominance rank, and aggression was more commonly directed towards subordinates and was heightened between individuals of adjacent rank, demonstrating that conflict over rank occurs in size-based hierarchies. Temperature did not affect overall levels or directionality/adjacency of aggression, but substantially altered subordinate growth patterns. In only the high temperature groups, growth rates of subordinates decreased as the size ratio between themselves and their immediate dominant approached 1.0, whilst growth rates of dominants were unaffected. This unique finding suggests that only under high temperatures subordinates may adopt growth regulation to resolve conflict, when the costs of conflict with dominants are greater. This provides the first causal link between abiotic stressors and changes to hierarchical structure and functioning, providing a springboard for further research into implications of temperature-dependent subordinate growth alteration at higher levels of ecological organisation.

Key words: **Dominance hierarchy; *Gambusia holbrooki;* growth regulation; climate change; temperature; conflict resolution; aggression**

INTRODUCTION

One of the most interesting questions in the field of animal behaviour is how the stability of social groups can be maintained despite the conflicts over limited resources (Sachs et al. 2004; Buston and Cant 2006; Ratnieks et al. 2006; Cant 2010). In many cases, a dominance hierarchy or "pecking order" forms, in which competitively superior dominants typically acquire a larger share of the resources than their subordinates, fuelling competition and conflict over rank (Clutton-Brock et al. 2001; Bergman et al. 2003; Valderrábano-Ibarra et al. 2007). From an evolutionary perspective, for groups to be maintained there must be mechanisms that determine how resources are shared, thereby resolving conflict and stabilising the hierarchy (Wiley and Rabenold 1984).

One of the most prominent theories explaining how conflict is resolved in social groups is here termed the 'threat-restraint' model. It suggests that higher ranked, dominant individuals target threats towards lower ranked subordinates, coercing cooperation (Clutton-Brock and Parker 1995; Cant and Johnstone 2009; Cant 2010). For a threat to be effective it must convey a significant fitness cost to the subordinate, and they most commonly take the form of increased aggression conveying the threat of injury or eviction from the group (Cant et al. 2001; Monnin et al. 2002; Buston 2003a; Heg et al. 2004a; Gilchrist 2006). Assuming that the outside options for the subordinate are limited (Emlen 1982; Wong et al. 2007; Buston and Zink 2009; Cant and Johnstone 2009), this offers a fitness incentive for subordinates to cooperate and appease dominants, resolving conflict and safeguarding their membership to the group (e.g. Bergmüller & Taborsky 2005). For example subordinates may cooperate by restraining their reproduction in order to avoid eviction or infanticide, as in cooperatively breeding meerkats, *Suricata suricatta* (Gilchrist

2006; Young et al. 2006; 2008), or to reduce mortality risks or loss of rank as in the queenless ant *Dinoponera quadriceps* (Monnin et al. 2002). Furthermore, subordinates may also resolve conflict by restraining their growth so they remain sufficiently smaller than their immediate dominants, which is evident in a number of social fish species (Buston 2003c; Heg et al. 2004b; Buston and Cant 2006; Wong et al. 2008; Ang and Manica 2010b). This mechanism relies on the ability of dominants to effectively convey threats and the ability of subordinates to both assess the threat and restrain themselves accordingly (Enquist et al. 1987; Neat et al. 1998a; Cant 2010). Therefore, if this mechanism were disrupted, conflict would not be resolved to the satisfaction of all individuals and the stability of the hierarchy may be undermined (Wiley and Rabenold 1984; Cowlishaw and Dunbar 1991; Alberts et al. 2003; Wong et al. 2007; Ang and Manica 2010a).

The focus of past research has predominantly centred on identifying the ultimate reasons for threats and restraint, and their influence on the stability of dominance hierarchies. Surprisingly, there have been no attempts to address how these mechanisms might respond to severe changes to the abiotic environment. This is important because the abiotic environment in which organisms live has changed and is changing rapidly, particularly in response to anthropogenic activities (Meehl et al. 2007). While there is an emerging literature demonstrating how abiotic factors such as temperature, climatic variability, water chemistry and environmental pollutants can directly and indirectly influence dominance interactions (reviewed in Wong *et al* 2012), there is a distinct lack of research investigating the link between abiotic variables and threats and restraint, and therefore hierarchy structure and stability. In light of future anthropogenic induced change it is imperative to

70 gain an understanding of how these mechanisms may be disrupted and how this may
71 influence higher levels of ecological organisation.

72 Social fishes can act as effective models for determining how hierarchies may respond to
73 abiotic perturbations, due to their formation of tractable size-based hierarchies and their
74 susceptibility to abiotic changes both in terms of behaviour, life history and development
75 (Jobling 1997; Sloman et al. 2001; Sokolowska and Kulczykowska 2009). Many fish species
76 form linear size-based hierarchies where the size asymmetries between group members
77 dictate the intensity of aggressive contests (Parker 1974; Enquist et al. 1987; Reddon et al.
78 2013). These hierarchies are often characterised by strict size-ratios between dominants
79 and subordinates (Buston 2003b; Heg et al. 2004b; Buston and Cant 2006; Wong et al. 2007;
80 Ang and Manica 2010b) where the threat of eviction from a dominant coerces subordinates
81 to regulate their growth to remain sufficiently smaller than their dominant (Buston 2003c;
82 Heg et al. 2004b; Wong et al. 2007; Wong et al. 2008). In these hierarchies, aggression
83 (dominant threat) and growth (subordinate restraint) together determine the success of
84 conflict resolution, and their potential alteration in response to changes to the abiotic
85 environment creates an exciting opportunity to assess the likely responses of dominance
86 hierarchies to changing abiotic conditions.

87 The Eastern mosquitofish, *Gambusia holbrooki,* is a freshwater poeciliid and is a highly
88 aggressive hierarchical species (Courtenay Jr et al. 1989; Bisazza and Marin 1991; Mills et al.
89 2004). It can tolerate a wide range of abiotic variables (Pyke 2005) allowing for a range of
90 environmental manipulations to be conducted. Although hierarchical structure has been
91 tentatively described as monarchistic amongst females (one large dominant and relatively
92 equal subordinates) (Caldwell and Caldwell 1962; Chen et al. 2011; Burns et al. 2012), there

93 has not been a definitive characterisation of hierarchy structure and the mechanisms
94 maintaining hierarchy stability in the species. Further, while previous studies have also
95 shown that increased temperature (from 10 to 35°C) leads to an increase in metabolic rate
96 (Cech Jr et al. 1980; Cech Jr et al. 1985), this has yet to be related to changes in growth rate
97 or hierarchy dynamics.

98 Using *Gambusia holbrooki* as a model species, we aimed to identify the effect of one
99 particular environmental factor, elevated temperature, on conflict resolution within
100 dominance hierarchies. Firstly, we hypothesised that groups of *G. holbrooki* form size-based
101 hierarchies in which conflict over rank is expressed by heightened aggression between
102 adjacent size-ranked individuals i.e. those more similar in size (Cant et al. 2006a; Wong et al.
103 2007; Ang and Manica 2010a). Secondly, we hypothesised that elevated temperature will
104 alter overall aggression levels and conflict over rank (i.e. aggression between adjacent
105 ranked individuals) within groups. Furthermore, we hypothesised that under elevated
106 temperature, growth rates of subordinates (but not of dominants) will be reduced or
107 "restrained" owing to the altered perceived conflict over rank with dominants and the
108 increasing metabolic costs of higher temperatures.

MATERIALS AND METHODS

Study Site and Species

Gambusia holbrooki were collected using handheld landing nets from a large freshwater pond located at the University of Wollongong, Australia (34.4°S 150.9°E) in March 2013. Individuals were then transported back to the laboratory in buckets. Each individual was sexed based on the shape of the anal fin (females: fan-shaped, males: modified spear-like fin, called a gonopodium (Pyke 2005)), measured using callipers (standard length (mm SL ± 0.1)) and weighed using an electronic balance. Individuals were uniquely tagged via injection of a coloured fluorescent elastomer (Northwest Technologies Inc.) in the dorsal musculature.

Five females and one male were randomly assigned to each of the twenty aquaria (60x30x30cm) in the laboratory. Although dominance behaviour within the female hierarchy was the focus of the study, a male was included in each group to more closely model wild populations. A 5:1 sex-ratio is within their natural range within the source population (Matthews, unpublished data) and is also consistent with previous studies reporting strongly female biased sex ratios (Tatara et al. 1999, 2002; Evans et al. 2003; Smith and Sargent 2006). Prior to introduction, each aquarium was lined with gravel and equipped with a filter, heater and 20cm length of PVC pipe used for shelter. Water in the tanks had been cycled and treated to remove chlorine and trace metals. Since the baseline temperature in the pond at the time of collection was 22.52 ± 0.39°C (mean ± SE), water was circulated at 22°C in the laboratory. Fish were allowed to acclimate to laboratory conditions for 1

month prior to the commencement of experimental manipulations, during which time they were fed daily with commercial fish flakes.

Experimental Design

Temperature manipulation

To assign treatment temperatures, pond water temperature was recorded daily for 1 month prior to commencement of the manipulations (March – April 2013). Maximum mean water and ambient temperatures were 22.71 ± 0.39°C and 25.26 ± 0.41°C respectively. Summer mean maximum temperatures in Wollongong are generally 3°C higher than March-April temperatures (i.e. approximately 26 – 28 °C) (BOM, 2012) and climate change predictions of 1.1 - 6.4°C increases by 2100 (Meehl et al. 2007) suggest that *G. holbrooki* pond populations will be subject to temperatures of over 30°C on an increasingly frequent basis. Therefore, after a 1-month acclimation period at 22°C, we increased the temperature to 32°C in the high temperature treatment groups (using 1°C per day increments (Nilsson et al. 2010; Seebacher et al. 2012)), to reflect a likely average maximum summer temperature predicted under an aggressive climate change model (Meehl et al. 2007). This created ten high temperature (32°C) treatment groups and ten control temperature (22°C) treatment groups (Figure 1).

Behavioural observations

In total, 4 observations were conducted over the course of the study (Figure 1). Observation 1 was conducted at the end of the 1 month acclimation period at 22°C to generate a baseline behavioural reference point. After this, the temperature was

153 raised in the ten high temperature treatment groups. Observation 2 was conducted
154 immediately after the temperature had reached 32°C and observation 3 was
155 conducted one month later (Figure 1). After this, the temperature in the treatment
156 groups was lowered back to 22°C (1°C decrease per day) and observation 4
157 conducted one month after the temperature had reached 22°C. Behaviours were
158 scored following an ethogram that we initially developed from pilot observations of
159 a random subset of 12 individuals over 5 days (Table 1). During each behavioural
160 observation, two groups were randomly selected and observed per day, making a
161 total of 10 days to observe all groups. One fish from within the group was randomly
162 selected and observed for 10 minutes, making a total of 60 minutes to observe all
163 individuals per group. These observations were conducted in the morning (09:00 –
164 11:00) and afternoon (13:00 - 15:00) to account for potential within-day variation
165 in behaviour. Although the frequency of all behaviours in the ethogram was scored,
166 the primary focus was to record the frequency of aggressive and submissive
167 behaviours between female group members to quantify dominance. In addition, we
168 focused on which individuals these behaviours were targeted, quantifying
169 "adjacency" (towards those of adjacent or non-adjacent rank) and "directionality"
170 (towards individuals of higher (dominant) or lower (subordinate) ranks).

171 In addition to behavioural traits, standard length (mm SL) and mass (g) of each
172 individual was measured after each round of observations, allowing the size rank of
173 each individual to be monitored and compared to their behavioural dominance rank
174 at each time point. It also enabled the calculation of percentage growth rate
175 (expressed as percentage increase in SL relative to initial size; Wong et al., 2007) to

monitor the effects of temperature and dominance interactions on individual growth rates. % growth was used because previous studies on social fishes has demonstrated that subordinates regulate their % growth rates in relation to the size ratio between themselves and their immediate dominants, and is therefore a means to detect conflict resolution within size-based hierarchies (Buston & Cant 2006; Wong et al 2007, 2008).

Throughout the course of the experiment a total of 4 females died in the treatment groups and 2 in control groups. In each of these cases, the female was the lowest ranked and smallest, causing the least disruption to the hierarchy structure. Additionally, 2 females in treatment groups and 3 in control groups underwent the process of masculinization (Krotzer 1990), whereby juvenile females mature as males. These individuals were removed from the group to ensure that only one male was present in each group at a time. Therefore, final sample sizes in terms of numbers of individuals were n = 35 (control) and n = 34 (high temperature).

Dominance Indices

Dominance indices were used to rank each individual based on the combination of aggressive and submissive behaviours. These rankings provide insight into how the hierarchy is organised (i.e. linear vs. monarchistic (Caldwell and Caldwell 1962; Chen et al. 2011). Specifically, David's scores (David 1987; David 1988) were used to quantify dominance ranks within groups. This index incorporates the relative strength (dominance) of each individual when quantifying dominance, and is currently the most suitable measure of the overall success or dominance of an

198 individual (Gammell et al. 2003; de Vries et al. 2006). This index defines each
199 dominance interaction (chase, bite etc.) as a contest which is simply won or lost
200 (aggression = win, submission = loss). The advantage of this method over others is
201 that dominance of an individual is determined by weighting each dyadic success
202 (contest win) by the unweighted estimate of the opponent's overall success. This
203 means that defeating a high-ranking individual is weighted more heavily than
204 defeating a low-ranking one and therefore minimises the chance of a highly
205 dominant individual skewing the index (de Vries et al. 2006).

206 To calculate David's score, the dyadic wins and losses for each individual were
207 tallied in a matrix where wins (aggressive behaviour given) were recorded in rows
208 and losses (aggressive behaviour received) were inversely recorded in columns. In
209 *Gambusia*, submissive behaviours are more subtle and harder to quantify than
210 aggressive displays (Matthews, personal observation), therefore it was more
211 accurate to solely assess aggressive acts and constitute the receipt of aggression as
212 a dyadic loss. Also, it is standard procedure to consider 'aggression received' as a
213 direct component of subordinate status in social fishes in general (Fitzpatrick et al.
214 2008). If an individual retaliated, both individuals received both a win and a loss. We
215 then calculated the dyadic proportions of wins: simply, the dyadic proportion of
216 wins for individual *i* in contests with individual *j* (P_{ij}) is the number of times *i* defeats
217 *j* (s_{ij}) divided by the total number of contests (n_{ij}), i.e. $P_{ij} = s_{ij} / n_{ij}$ (David 1987; David
218 1988; de Vries et al. 2006).

219 Using the formula for David's score:

220 $$DS = w + w_2 - l - l_2$$

221 where w represents the sum of i's P_{ij} values, i.e. $w = \sum P_{ij}\ (j = 1....N;\ j \neq i)$; w_2
222 represents a weighted sum of i's P_{ij} values (weighted by the w values of its
223 opponents), i.e. $w_2 = w_j P_{ij}\ (j = 1....N;\ j \neq i)$; l represents the sum of i's P_{ji} values, (i.e. l
224 $= \sum P_{ji}\ (j = 1....N;\ j \neq i)$); and l_2 represents a weighted sum of i's P_{ji} values (weighted by
225 the l values of its opponents), i.e. $l_2 = \sum l_j P_{ji}\ (j = 1....N;\ j \neq i)$ (David 1988, p. 108; de
226 Vries et al. 2006). David's scores were then normalised (see de Vries et al. 2006) and
227 each group member assigned an ordinal rank based on their score. This assigned
228 rank was then used in analyses to investigate to assess whether standard length
229 was a suitable proxy indicator for dominance in *G. holbrooki.*

230 ***Statistical Analysis***

231 Aggression data from am and pm observations was combined for each individual
232 because there were no significant differences in aggression between observations
233 (Paired t-test: $t_{99} = 0.5383$, $p = 0.5916$), hence all analysis of aggression use these
234 combined behaviours. For all analyses using aggression data, Generalized Estimating
235 Equations (GEE's) (negative binomial distribution with logit link function) were used
236 to account for repeated measures (four temporal observations), covariates (size,
237 size-ratio) and random effects (Group ID) as well as the non-normally distributed
238 aggression data (counts with large variance). Linear mixed models (LME's) were
239 used for analysis of growth rates and size as they were normally distributed. All
240 analyses were conducted using IBM SPSS Statistics Data Editor V21.

241 To investigate whether individuals form size-based dominance hierarchies within
242 groups, dominance ranks (based on Normalised David's Scores) were compared to
243 individual body size using a Linear mixed model (LME). This model accounted for
244 time (four repeated measurements) and treatment, enabling us to test whether the
245 relationship between size and dominance was consistent over time and resilient to
246 manipulation (i.e. altered temperature and disturbing fish due to obtain size
247 measures).

248 Conflict over rank is expressed as a combination of both aggressive suppression
249 from dominants (higher ranks) and dominance testing by subordinates (lower ranks)
250 and therefore its prevalence is indicated by heightened aggression between
251 individuals of adjacent size-rank (Cant et al. 2006b). To investigate this, each group
252 member was assigned a size-rank and aggression was categorised by adjacency (i.e.
253 whether it was directed towards or received by adjacent or non-adjacent size
254 ranks). Since there are more non-adjacent group members, aggression counts were
255 divided by the number of non-adjacent group members (either 2 or 3 depending on
256 the focal individual's size rank). Similarly, the frequency of aggression given to and
257 received from adjacent ranked group members was divided by the number of
258 adjacent ranked group members (either 1 or 2). To investigate the relative
259 contributions of aggressive suppression by dominants and dominance testing by
260 subordinates to overall conflict over rank, aggression data was further categorised
261 by its directionality i.e. towards or from dominants (higher size rank) or
262 subordinates (lower size rank) group members. The size ratio between dominants
263 and subordinates (calculated as SL of subordinate / SL of immediate dominant;

264 Wong et al., 2007; 2008) was also included in the model to investigate whether
265 aggression escalated as subordinates approached the size of dominants.

266 To determine the potential effects of increased temperature on growth, we firstly
267 compared the growth response of individuals over time between treatment and
268 control groups using an LME incorporating time, treatment, size rank, dominance
269 rank, aggression received as well as the random effects of group ID. To investigate
270 whether growth rates of both subordinates and dominants were related to the size
271 ratio with their immediate dominants/subordinates, and whether this relationship
272 was affected by elevated temperature, we performed another LME incorporating
273 treatment, size rank, dominance rank, aggression received as well as the random
274 effects of group ID.

RESULTS

Is there a size based hierarchy and conflict over rank?

The standard length (SL) of individuals was significantly related to their dominance rank (DR: based on normalised David's scores) (LME, SL: $F_{1,99} = 41.51$, $P < 0.0001$), supporting the hypothesis that size-based dominance hierarchies exist amongst females in *G. holbrooki* groups (Figure 2). There were no significant interactions between SL and either time or treatment on dominance rank, demonstrating that size-based hierarchies were consistent over time and altered temperatures (SL*Time: $F_{3,88.} = 0.06$, $P = 0.98$; SL*Treatment: $F_{1,100} = 0.62$, $P = 0.43$). Furthermore DR was not affected by treatment (Treatment: $F_{1,100.} = 0.81$, $P = 0.37$) nor did it vary significantly between observations (Time: $F_{3,88} = 0.04$, $P = 0.99$), demonstrating that the exact ranks occupied by individuals were consistent throughout the experiment.

To investigate whether there is conflict over rank within the size-based hierarchy, we compared the amount of aggression directed toward adjacent versus non-adjacent size-ranked group members. As predicted, the rate of aggression between adjacent size-ranked individuals was significantly greater than between non-adjacent size-ranked individuals (GEE, Adjacency: Wald $\chi^2_{df=1} = 7.27$, $P = 0.007$) (Figure 3) and there was no significant interaction between time and adjacency (Time*Adjacency: Wald $\chi^2_{df=3} = 5.03$, $P = 0.17$) (Figure 4), demonstrating that the effect of adjacency was consistent between observations. However, the size ratio between a subordinate and its immediate dominant was not significantly related to

296 the amount of aggression directed towards them by their immediate dominant
297 (Wald $\chi^2_{df=1} = 0.646$, $P = 0.422$).

298 In terms of directionality, aggression given was more often directed from dominants
299 towards subordinates rather than subordinates to dominants (GEE, Directionality:
300 Wald $\chi^2_{df=1} = 36.43$, $P < 0.0001$) (Figure 3). Furthermore, this effect was more
301 pronounced when between non-adjacent rather than adjacent size-ranked
302 individuals (Adjacency*Directionality: Wald $\chi^2_{df=1} = 6.35$, $P = 0.012$) (Figure 3) and
303 was not affected by time (Directionality*Time: Wald $\chi^2_{df=3} = 2.37$, $P = 0.50$). There
304 was also a significant interaction between adjacency and dominance rank
305 (Adjacency*Dominance Rank: Wald $\chi^2_{df=4} = 17.16$, $P = 0.002$) with higher dominance
306 ranks contributing more to both overall aggression and aggression targeted toward
307 adjacent size ranks (Figure 5). Importantly, the effect of adjacency was still
308 significant when rank 1 dominants were excluded from the analysis (Wald $\chi^2_{df=1} =$
309 5.52, $P = 0.019$), indicating that conflict over rank is exhibited throughout the
310 hierarchy.

311 ***Does temperature influence threats and restraint?***

312 **Effect on Threats**

313 Average rates of aggression were significantly higher within treatment groups
314 compared to control groups (GEE, Treatment: Wald $\chi^2_{df=1} = 4.394$, $P = 0.036$) (Figure
315 6). While this is suggestive of a temperature effect, further exploration of the data
316 revealed that levels of aggression were significantly higher in treatment groups
317 even during observation 1 when all these groups were maintained at the control

318 temperature (Wald $\chi^2_{df=1}$ = 7.94, P = 0.005) (Figure 6), demonstrating that
319 individuals in treatment groups had an intrinsically higher aggression rate than
320 control groups. In support of this conclusion, we found no significant interaction
321 between treatment and time (Treatment*Time: Wald $\chi^2_{df=3}$ = 0.19, P = 0.98) nor was
322 there any effect of time on aggression levels (Time: Wald $\chi^2_{df=3}$ = 5.1, P = 0.16).
323 Therefore, temperature did not affect overall levels of aggression exhibited in
324 groups of *G. holbrooki.* Furthermore, there was no effect of treatment on conflict
325 over rank (Treatment*Adjacency: Wald $\chi^2_{df=1}$ = 0.75, P = 0.39), suggesting that
326 treatment conditions did not have an effect on conflict over rank in terms of
327 threats.

328 **Effect on Restraint**

329 To determine whether growth was affected by temperature, percentage growth
330 rates were compared between high temperature and control groups across the four
331 observations. Growth rates in treatment groups was significantly lower than in
332 control groups (LME: Treatment: $F_{1,88}$ = 53.3, P <0.0001). Whilst growth rates did not
333 significantly change over time (Time: $F_{2,88}$ = 1.0, P = 0.37), the difference in growth
334 rates between treatment and control groups declined over time (Time*Treatment:
335 $F_{2,88}$ = 6.7, P = 0.002) (Figure 7), suggesting that individuals acclimated to higher
336 temperatures in terms of overall growth rates.

337 To determine if temperature influenced whether subordinates regulate their
338 growth to resolve conflict with their immediate dominant, the relationship between
339 percentage growth rates (change in SL between observation 1 and 3 only) and initial

size ratios between dominants and subordinates (observation 1 only) were investigated. This relationship was investigated for both the size ratio between an individual and its immediate dominant (DomSR: Figure 8a) and its immediate subordinate (SubSR: Figure 8b) for both treatment and control conditions. Note that observation 4 was excluded in the calculation of growth rates because the temperature experienced by treatment groups was reduced back to control levels at this point, potentially diluting any effects of elevated temperature.

When comparing an individual's growth to the size ratio with its immediate dominant (i.e. as a subordinate), there was a significant interaction between treatment and DomSR (Treatment*DomSR: $F_{1,56}$ = 5.0, P = 0.030) (Figure 8a) after controlling for the average rate of aggression received ($F_{1,35}$ = 0.03, P = 0.85) and body size of the individual ($F_{1,56}$ = 2.4, P = 0.076). Importantly, there was no interaction between treatment and body size (Treatment*Size: $F_{1,56}$ = 0.25, P = 0.86) meaning that the differences in growth rate patterns between treatment groups were not affected by the size of the individual. Specifically, SubGR was negatively correlated with DomSR in treatment groups only (R^2 = 0.24 P = 0.003), whereas there was no relationship between growth rates and DomSR in control groups (R^2 = 0.01 P = 0.57) (Figure 8a). Overall, SubGR declined if they became more similar in size to their immediate dominant (i.e. as DomSR approaches 1), yet this was only evident at elevated temperatures. The influence of the initial size ratio between subordinates and their immediate dominants on subordinate growth rates was only evident at elevated temperatures. Importantly this pattern was independent of body size and size rank.

In contrast, there was no interaction between Treatment and SubSR (Treatment*SubSR: $F_{1,56}$ = 0.409, P = 0.53) (Figure 8b), after controlling for the average rate of aggression received ($F_{1,66}$ = 0.24, P = 0.63) and body size of the individual ($F_{1,68}$ = 9.8, P < 0.01). Although there appears to be a positive relationship between individual growth rates and SubSR (as it approaches 1), this relationship was not statistically significant for either control ($F_{1,28}$ = 2.5, P = 0.13) or the high temperature treatment ($F_{1,27}$ = 2.7, P = 0.11) (Figure 8b).

DISCUSSION

Hierarchy Structure

The first goal of this study was to quantify the baseline structure of dominance hierarchies in *G. holbrooki*. Within-group dominance ranks (based on David's scores) were positively related to individual standard length, supporting the assertion that *G. holbrooki* forms size-based hierarchies within groups. The relationship between dominance and size did not differ between treatment and control groups or between successive observations, emphasizing the inherent nature of size-related dominance relationships within groups of *G. holbrooki*. This finding is contrary to a previous quantification of the hierarchy by Chen *et al.* (2011), who used male mate preference as a proxy for dominance amongst females . However, Chen et al (2011) used an indirect approach of assessing hierarchy structure, and no statistical analysis was performed to assess whether female dominance rank was related to body size, as in the current study. In some cases, disturbances of hierarchical groups such as the removal of individuals for measurements have been shown to disrupt rank order (Dugatkin et al. 1994; Chase et al. 2002). However, in our study DR did not change significantly after the observational disturbances, further emphasising the inherent and replicable nature of the size based hierarchy structure. Therefore, we conclude that size is a strong indicator of dominance rank in *G. holbrooki*, as has been shown for a wide range of other social fishes (Forrester 1991; Webster 2004; Whiteman and Côté 2004; Ward et al. 2006).

More aggression was observed between individuals of adjacent compared to non-adjacent size-rank, indicating that individuals typically target aggression towards conspecifics that are

more similar to themselves in size. This discovery indicates that conflict over rank occurs in social groups of *G. holbrooki* and again highlights the importance of body size for the structure of these hierarchies. Intuitively, more aggression was directed towards subordinates rather than dominants, and this was more evident for non-adjacent ranks (where size differences are larger). This indicates that aggression within groups primarily constitutes dominant suppression of subordinates rather than dominance testing by subordinates (Cant et al. 2006b). Although average rates of aggression were higher for larger, more dominant individuals (Cant et al. 2006b)(Figure 5), conflict over rank was still prevalent for all individuals down the hierarchy. This is contrary to a previous paradigm that described the hierarchical structure of *G. holbrooki* groups as "monarchistic" (Caldwell and Caldwell 1962; Chen et al. 2011), where one supreme dominant presides over a group of relatively equally ranked subordinates. Although a greater proportion of aggression was given by the larger, more dominant individuals rather than by the smaller, more subordinate individuals, it would be misleading to conclude a monarchistic structure in this case because conflict over rank still permeated throughout the entire group.

Although we found that adjacent size-ranked subordinates received more aggression from their immediate dominants than non-adjacent ranked subordinates, the size ratio between subordinates and their immediate dominant did not have an effect on the amount of aggression received. This is contrary to our expectation that there may be a specific size-ratio threshold of tolerance, which if exceeded would attract heightened aggression from dominants, as seen in other social fishes (Wong et al. 2007; Ang and Manica 2010a). Even so, our results still indicate that aggression is heightened between individuals of similar size (i.e. adjacent size ranks) which is consistent with the threat-restraint model of hierarchy

stability (Buston 2003c; Buston and Cant 2006; Wong et al. 2007; Cant and Johnstone 2009; Cant 2010) and the literature regarding the role of size asymmetries in determining the outcome, duration and the intensity of pair-wise contests (Parker 1974; Enquist et al. 1987; Reddon et al. 2011).

The necessity for continual assessment and re-assessment (after perturbations) of an individual's position in a hierarchy is explored by many contemporary conflict resolution models (threat-restraint (Cant 2010), reproductive skew (Kokko 2003), and pay-to-stay (Cockburn 1998; Kokko et al. 2002)). Our results show that conflict over rank (the pattern of heightened aggression between individuals of adjacent size ranks) and dominance ranks were highly consistent over time and thus individual social relationships were resilient to both experimental (temperature treatment) and observational (handling, measuring and water changes) disturbances. These are important findings as perturbations such as changes in water levels and experimental removal have been shown to disrupt dominance cues and destabilise hierarchies (Dugatkin et al. 1994; Sloman et al. 2001; Chase et al. 2002; Sloman et al. 2002; Gonçalves-de-Freitas et al. 2008), often inducing a re-assessment phase whereby heightened aggression is necessary for individuals to re-assess their place within the hierarchy (Ang and Manica 2010a; Wong and Balshine 2011). In *G. holbrooki,* the hierarchy structure did not appear disrupted, minimising the need for re-assessment hence reducing fitness costs (injury and metabolic requirements) to all group members.

Temperature Effects

Effect on Aggression

The second goal of this study was more explicitly to investigate the effects of increased temperature on conflict over rank and the factors that stabilise the hierarchy. Overall rates of aggression were higher in treatment than control groups, however this was not due to the effect of elevated temperature because aggression in treatment groups was naturally higher even during observation 1 when all groups were maintained at the control temperature. Furthermore, as there was no significant interaction between time and treatment, this pattern was consistent on a temporal scale suggesting that individuals within treatment groups had an intrinsically higher rate of aggression. While the potential role of observer bias must be considered to account for these discrepancies, the randomized division of groups into treatment and control conditions was made after the first observation was conducted, making observer bias highly unlikely. Instead, these results suggest that the intrinsically higher aggression rate of treatment groups was simply due to chance, leading to the conclusion that elevated temperature had no effect on overall aggression rates. Furthermore, as stated earlier, increased temperature did not affect the adjacency or directionality leading to the conclusion that temperature did not alter conflict over rank in terms of changes in aggressive displays.

Effect on restraint – Growth

As outlined above, the use of threats in the form of aggression between adjacent ranks was not elevated in response to increased temperature. However, the alternative mechanism of conflict resolution, namely subordinate growth regulation, may have been adopted to

457 counteract the increased fitness costs of aggression under increased thermal stress
458 (Metcalfe et al. 1995; Neat et al. 1998b). Whilst aggression is costly to dominants in terms of
459 metabolic activity, the threat of injury for subordinates (Neat et al. 1998b; Sinclair et al.
460 2011) combined with the increased metabolic load of higher temperatures means there
461 may be a fitness pay-off for subordinates to restrain their growth and thus avoid being the
462 target of aggression. Smaller and therefore subordinate individuals however, may
463 experience a steeper increase in the metabolic costs of increased temperature and
464 therefore may exhibit lower growth rates than bigger individuals. Alternatively or
465 additionally, individuals may maintain certain levels of aggression but as a result, suffer
466 costs in terms of growth rates under high temperatures. Therefore the effect of
467 temperature on subordinate growth rates was investigated (whilst controlling for the
468 individual size) to determine what effect, if any, temperature may have on growth rate
469 alterations as a mechanism of conflict resolution within hierarchies (Heg et al. 2004b;
470 Buston and Cant 2006; Wong et al. 2007; Wong et al. 2008).

471 With respect to average rates of growth, increased temperatures caused an overall
472 decrease in the growth rates of *G. holbrooki*. However, this negative impact was reduced
473 over time, suggesting that individuals acclimated to higher temperatures in relation to
474 growth rates in the treatment groups. These findings are consistent with a previous study
475 showing that individuals grew faster and larger at lower (25°C) compared to higher (32°C)
476 temperatures (Meffe 1992). Given that individuals in our study were first acclimated to
477 22°C, the initial decline in growth rates was expected since metabolic requirements increase
478 with temperature (Elliott 1976; Fonds et al. 1992; Jobling 1997; Gillooly et al. 2001) and food
479 availability was not increased to match these requirements. Previous studies have shown

480 that *Gambusia* species (*G. holbrooki* and *G. affinis*) can increase their upper limits of
481 thermal tolerance after acclimation to high temperatures (Otto 1973; Cherry et al. 1976;
482 Grigaltchik et al. 2012; Seebacher et al. 2012) allowing them to adapt to and often dominate
483 harsh environmental conditions (Pyke 2005; Pyke 2008). This ability could partially explain
484 the acclimation responses with respect to growth witnessed in this study, as well as
485 facilitate their competitive dominance throughout many habitats and environments
486 worldwide (Grigaltchik et al. 2012).

487 Despite this overall growth depression, there were more subtle effects of elevated
488 temperature on the growth rates of individuals. For treatment groups, it was found that
489 subordinate growth rate significantly decreased as the initial size ratio with their immediate
490 dominant increased; in contrast for control groups, there was no relationship between
491 subordinate growth rates and initial size ratio. This result suggests that subordinates may
492 regulate their growth in relation to the size ratio between themselves and their immediate
493 dominant, but only under high temperatures. As such, conflict over rank may be heightened
494 under increased temperature, but it is expressed and resolved through the commencement
495 of growth regulation by subordinates rather than through an increased aggression from
496 dominants.

497 During normal conditions, the costs of injury are less severe and subordinates can afford to
498 grow larger and "challenge" dominants. Although it may intuitively seem that reduced
499 growth of subordinates may reflect an inferior ability to cope with increased temperatures,
500 our results show that there was no interactive effect between body size and treatment on
501 subordinate growth rates. What we found instead was that the reduction in subordinate
502 growth was modulated by the size ratio with their immediate dominant and this pattern was

consistent for all members of the size hierarchy. Importantly, this pattern also still held after controlling for the inherently higher rates of aggression received by an individual in treatment groups, indicating that the growth regulation response by subordinates was driven by the elevated temperature *per se* and not an increase in aggression from dominants.

Dominant aggression has long been assumed as the primary mechanism by which hierarchies are stabilised (Reeve 1992; Balshine-Earn et al. 1998; Ang and Manica 2010a) and indeed, previous research has shown that in poeciliid fishes, growth and maturation of subordinates can be inhibited by dominants (Borowsky 1973, 1987). While we do not dismiss a role of dominant aggression, our results more strongly support the alternative adaptive perspective that subordinates can restrain or regulate their growth (reproduction and/or behaviour) in order to resolve conflict (Johnstone and Cant 1999; Heg et al. 2004b; Wong et al. 2007; Wong et al. 2008; Cant 2010). Importantly, we show that subordinate growth rate is negatively correlated with increasing size ratio between subordinates and their immediate dominant whilst the growth rate of dominants remains stable under high temperatures, suggesting this pattern was not a by-product of simple metabolic alterations under high temperature. Furthermore, if the reduction in subordinate growth rate was due to dominant inhibition (Borowsky 1973, 1987) rather than subordinate regulation, we would expect that the decrease in subordinate growth rate with increasing size ratio would be mirrored by an increase in dominant aggression with increasing size ratio in treatment groups, which was not the case. Finally, the negative correlation between subordinate growth rate and initial size ratio under high temperatures held true after statistically controlling for the amount of aggression imposed by dominants and the size of the

526 subordinate. Therefore, our data suggests that the most parsimonious explanation is
527 subordinate growth restraint, with the novel twist that subordinate growth restraint is itself
528 influenced by abiotic factors. Further research focusing directly on quantifying subordinate
529 food consumption (Wong et al. 2008), metabolic costs of aggression (Metcalfe et al. 1995;
530 Neat et al. 1998b) dominance inhibition (Borowsky 1973, 1987) and the social consequences
531 of non-compliant subordinates (e.g. eviction) (Wong et al. 2007) would now be important
532 to further strengthen our conclusions.

533 The suggestion of subordinate growth regulation (and perhaps some degree of dominance
534 inhibition) indicates that although aggression between adjacent ranked individuals did not
535 increase, conflict over rank does in fact increase at higher temperatures but it is expressed
536 through either voluntary or forced reduction in growth rate. One reason for the increased
537 conflict could relate to the increased metabolic costs of living at higher temperatures (Cech
538 Jr et al. 1980; Cech Jr et al. 1985; Gillooly et al. 2001). Throughout the experiment, food
539 availability was controlled for and not elevated to match increasing metabolic requirements,
540 rendering food a limited resource at high temperatures. Furthermore, size and social rank
541 are important determinants of food consumption in dominance hierarchies (Forrester
542 1991), and food is known to be a key limiting factor of female fecundity in fishes and other
543 animals (Wasser and Barash 1983; Berglund et al. 1993; Clutton-Brock et al. 1998; Ali and
544 Wootton 1999; Wong et al. 2008) generating more intense conflict over rank at high
545 temperatures. Thus, at higher temperatures, conflict over limited food resources and hence
546 conflict over rank may be intensified but expressed through the commencement of
547 subordinate growth regulation rather than through costly aggressive behaviours.

548 Although groups of *G. holbrooki* can be large and freely mixing in the wild (Pyke 2005; Pyke
549 2008), groups generally swim in small well separated groups (McPeek 1992; Bisazza and
550 Marin 1995) and small isolated groups are frequently found puddles, small ponds and
551 waterways (Matthews, unpublished observation). Presumably, this would apply some
552 degree of selective pressure on individuals to form size-based hierarchies and adopt
553 strategies for conflict resolution, even though groups may subsequently mix with other
554 groups and dominance hierarchies re-established. There is however, limited literature
555 regarding the social structure of *G. holbrooki* in the wild, hence future research would be
556 invaluable for investigating the complexities and variations of social size hierarchies and for
557 confirming dominance relationships in this species under more natural conditions.

558 To conclude, our results demonstrate that there is a high degree of hierarchy structure
559 within groups of female *G. holbrooki*. Under elevated temperatures, subordinates are not
560 subjected to higher rates of aggression, rather, their growth rate declines such that they
561 avoid becoming too similar in size to their immediate dominant. This suggests that conflict
562 over rank does increase with temperature, possibly because of increased metabolic costs
563 and therefore greater competition for food resources. Growth restraint may be an
564 appropriate strategy to avoid the fitness costs of elevated aggression (injury, mortality and
565 eviction), especially because of the already increased metabolic demands of elevated
566 temperatures (Elliott 1976; Gillooly et al. 2001). This is the first empirical study to show that
567 growth regulation as a mechanism to resolve conflict is conditional on abiotic conditions,
568 providing an important foundation for further research exploring the impacts of abiotic
569 variables on conflict over rank, hierarchy structure and hierarchy stability in other social
570 species.

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572 **REFERENCES**

573 Alberts SC, Watts HE, Altmann J. 2003. Queuing and queue-jumping: long-term patterns of
574 reproductive skew in male savannah baboons, Papio cynocephalus. Anim Behav. 65:821-840.
575 Ali M, Wootton R. 1999. Effect of variable food levels on reproductive performance of breeding
576 female three-spined sticklebacks. J Fish Biol. 55:1040-1053.
577 Ang TZ, Manica A. 2010a. Aggression, segregation and stability in a dominance hierarchy. Proc R Soc
578 B-Biol Sci. 277:1337-1343.
579 Ang TZ, Manica A. 2010b. Unavoidable limits on group size in a body size-based linear hierarchy.
580 Behav Ecol. 21:819-825.
581 Balshine-Earn S, Neat FC, Reid H, Taborsky M. 1998. Paying to stay or paying to breed? Field
582 evidence for direct benefits of helping behavior in a cooperatively breeding fish. Behav Ecol.
583 9:432-438.
584 Berglund A, Magnhagen C, Bisazza A, Konig B. 1993. Female-female competition over reproduction.
585 Behav Ecol. 4:184-187.
586 Bergman TJ, Beehner JC, Cheney DL, Seyfarth RM. 2003. Hierarchical classification by rank and
587 kinship in baboons. Science. 302:1234-1236.
588 Bergmüller R, Taborsky M. 2005. Experimental manipulation of helping in a cooperative breeder:
589 helpers 'pay to stay' by pre-emptive appeasement. Anim Behav. 69:19-28.
590 Bisazza A, Marin G. 1991. Male size and female mate choice in the eastern mosquitofish (*Gambusia*
591 *holbrooki*: Poeciliidae). Copeia.730-735.
592 Bisazza A, Marin G. 1995. Sexual selection and sexual size dimorphism in the eastern mosquitofish
593 *Gambusia holbrooki* (Pisces Poeciliidae). Ethol Ecol Evol. 7:169-183.
594 Borowsky RL. 1973. Social control of adult size in males of *Xiphophorus variatus*.
595 Borowsky RL. 1987. Agonistic behavior and social inhibition of maturation in fishes of the genus
596 *Xiphophorus* (Poeciliidae). Copeia.792-796.

Burns AL, Herbert-Read JE, Morrell LJ, Ward AJ. 2012. Consistency of leadership in shoals of mosquitofish (*Gambusia holbrooki*) in novel and in familiar environments. PloS one. 7:e36567.

Buston PM. 2003a. Forcible eviction and prevention of recruitment in the clown anemonefish. Behav Ecol. 14:576-582.

Buston PM. 2003b. Mortality is associated with social rank in the clown anemonefish (*Amphiprion percula*). Mar Biol. 143:811-815.

Buston PM. 2003c. Size and growth modification in clownfish. Nature. 424:145-146.

Buston PM, Cant MA. 2006. A new perspective on size hierarchies in nature: patterns, causes, and consequences. Oecologia. 149:362-372.

Buston PM, Zink AG. 2009. Reproductive skew and the evolution of conflict resolution: a synthesis of transactional and tug-of-war models. Behav Ecol. 20:672-684.

Caldwell MC, Caldwell DK. 1962. Monarchistic dominance in small groups of captive mosquitofish *Gambusi affinis patruelis*. Bull South Calif Acad Sci. 61:37-43.

Cant M, English S, Reeve H, Field J. 2006a. Escalated conflict in a social hierarchy. Proc R Soc Lond, Ser B: Biol Sci. 273:2977-2984.

Cant MA. 2010. The role of threats in animal cooperation. Proc R Soc Lond, Ser B: Biol Sci.

Cant MA, Johnstone RA. 2009. How threats influence the evolutionary resolution of within-group conflict. Am Nat. 173:759-771.

Cant MA, Llop JB, Field J. 2006b. Individual variation in social aggression and the probability of inheritance: theory and a field test. Am Nat. 167:837-852.

Cant MA, Otali E, Mwanguhya F. 2001. Eviction and dispersal in co-operatively breeding banded mongooses (*Mungos mungo*). J Zool. 254:155-162.

Cech Jr J, Massingill M, Wragg T, Grant C, The food demands of mosquitofish, *Gambusia affinis*. Proceedings and papers of the Forty-eighth Annual Conference of the California Mosquito

622 and Vector Control Association, Inc January 20-23, 1980 Quality Inn, Anaheim,
623 California1980. CMVCA Press. p. 45-47.
624 Cech Jr JJ, Massingill MJ, Vondracek B, Linden AL. 1985. Respiratory metabolism of mosquitofish,
625 Gambusia affinis: effects of temperature, dissolved oxygen, and sex difference. Environ Biol
626 Fishes. 13:297-307.
627 Chase ID, Tovey C, Spangler-Martin D, Manfredonia M. 2002. Individual differences versus social
628 dynamics in the formation of animal dominance hierarchies. Proc Natl Acad Sci. 99:5744-
629 5749.
630 Chen T, Beekman M, Ward AJW. 2011. The role of female dominance hierarchies in the mating
631 behaviour of mosquitofish. Biol Lett. 7:343-345.
632 Cherry DS, Rodgers Jr JH, Cairns Jr J, Dickson KL, Guthrie RK. 1976. Responses of mosquitofish
633 (*Gambusia affinis*) to ash effluent and thermal stress. Trans Am Fish Soc. 105:686-694.
634 Clutton-Brock T, Brotherton P, Smith R, McIlrath G, Kansky R, Gaynor D, O'riain M, Skinner J. 1998.
635 Infanticide and expulsion of females in a cooperative mammal. Proc R Soc B-Biol Sci.
636 265:2291-2295.
637 Clutton-Brock TH, Brotherton PNM, Russell AF, O'Riain MJ, Gaynor D, Kansky R, Griffin A, Manser M,
638 Sharpe L, McIlrath GM, Small T, Moss A, Monfort S. 2001. Cooperation, Control, and
639 Concession in Meerkat Groups. Science. 291:478-481.
640 Clutton-Brock TH, Parker GA. 1995. Punishment in animal societies. Nature. 373:209-216.
641 Cockburn A. 1998. Evolution of helping behavior in cooperatively breeding birds. Annu Rev Ecol
642 Syst.141-177.
643 Courtenay Jr W, Meffe G, Snelson Jr F. 1989. Small fishes in strange places: a review of introduced
644 poeciliids. Ecology and evolution of livebearing fishes (Poeciliidae).319-331.
645 Cowlishaw G, Dunbar RIM. 1991. Dominance rank and mating success in male primates. Anim Behav.
646 41:1045-1056.

David HA. 1987. Ranking from unbalanced paired-comparison data. Biometrika. 74:432-436.

David HA, 1988. The method of paired comparisons: C. Griffin.

de Vries H, Stevens JM, Vervaecke H. 2006. Measuring and testing the steepness of dominance hierarchies. Anim Behav. 71:585-592.

Dugatkin LA, Alfieri MS, Moore AJ. 1994. Can Dominance Hierarchies be Replicated? Form‐re‐form Experiments using the Cockroach (*Nauphoeta cinerea*). Ethology. 97:94-102.

Elliott J. 1976. The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta L.*) in relation to body weight, water temperature and ration size. The Journal of Animal Ecology.923-948.

Emlen ST. 1982. The evolution of helping. I. An ecological constraints model. Am Nat.29-39.

Enquist M, Ljungberg T, Zandor A. 1987. Visual assessment of fighting ability in the cichlid fish *Nannacara anomala*. Anim Behav. 35:1262-1264.

Evans JP, Pierotti M, Pilastro A. 2003. Male mating behavior and ejaculate expenditure under sperm competition risk in the eastern mosquitofish. Behav Ecol. 14:268-273.

Fitzpatrick JL, Desjardins JK, Milligan N, Stiver KA, Montgomerie R, Balshine S. 2008. Female-mediated causes and consequences of status change in a social fish. Proc R Soc B-Biol Sci. 275:929-936.

Fonds M, Cronie R, Vethaak AD, Van Der Puyl P. 1992. Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. Neth J Sea Res. 29:127-143.

Forrester GE. 1991. Social rank, individual size and group composition as determinants of food consumption by humbug damselfish, *Dascyllus aruanus*. Anim Behav. 42:701-711.

Gammell MP, de Vries H, Jennings DJ, Carlin CM, Hayden TJ. 2003. David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. Anim Behav. 66:601-605.

671 Gilchrist JS. 2006. Female eviction, abortion, and infanticide in banded mongooses (*Mungos mungo*):
672 implications for social control of reproduction and synchronized parturition. Behav Ecol.
673 17:664-669.
674 Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL. 2001. Effects of size and temperature on
675 metabolic rate. Science. 293:2248-2251.
676 Gonçalves-de-Freitas E, Teresa FB, Gomes FS, Giaquinto PC. 2008. Effect of water renewal on
677 dominance hierarchy of juvenile Nile tilapia. Appl Anim Behav Sci. 112:187-195.
678 Grigaltchik VS, Ward AJ, Seebacher F. 2012. Thermal acclimation of interactions: differential
679 responses to temperature change alter predator–prey relationship. Proc R Soc B-Biol Sci.
680 279:4058-4064.
681 Heg D, Bachar Z, Brouwer L, Taborsky M. 2004a. Predation risk is an ecological constraint for helper
682 dispersal in a cooperatively breeding cichlid. Proc R Soc B-Biol Sci. 271:2367-2374.
683 Heg D, Bender N, Hamilton I. 2004b. Strategic growth decisions in helper cichlids. Proc R Soc B-Biol
684 Sci. 271:S505-S508.
685 Jobling M. 1997. Temperature and growth: modulation of growth rate via temperature change.
686 Seminar series - society for experimental biology Cambridge University Press. p. 225-254.
687 Johnstone RA, Cant MA. 1999. Reproductive skew and the threat of eviction: a new perspective. Proc
688 R Soc Lond, Ser B: Biol Sci. 266:275-279.
689 Kokko H. 2003. Review Paper: Are reproductive skew models evolutionarily stable? Proc R Soc Lond,
690 Ser B: Biol Sci. 270:265-270.
691 Kokko H, Johnstone RA, Wright J. 2002. The evolution of parental and alloparental effort in
692 cooperatively breeding groups: when should helpers pay to stay? Behav Ecol. 13:291-300.
693 Krotzer MJ. 1990. The effects of induced masculinization on reproductive and aggressive behaviors
694 of the female mosquitofish, *Gambusia affinis affinis*. Environ Biol Fishes. 29:127-134.

McPeek MA. 1992. Mechanisms of sexual selection operating on body size in the mosquitofish (*Gambusia holbrooki*). Behav Ecol. 3:1-12.

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A. 2007. Global climate projections. Climate change.747-845.

Meffe GK. 1992. Plasticity of Life-History Characters in Eastern Mosquitofish (*Gambusia holbrooki*: Poeciliidae) in Response to Thermal Stress. Copeia. 1992:94-102.

Metcalfe NB, Taylor AC, Thorpe JE. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Anim Behav. 49:431-436.

Mills MD, Rader RB, Belk MC. 2004. Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. Oecologia. 141:713-721.

Monnin T, Ratnieks FL, Jones GR, Beard R. 2002. Pretender punishment induced by chemical signalling in a queenless ant. Nature. 419:61-65.

Neat FC, Huntingford FA, Beveridge M. 1998a. Fighting and assessment in male cichlid fish: the effects of asymmetries in gonadal state and body size. Anim Behav. 55:883-891.

Neat FC, Taylor AC, Huntingford FA. 1998b. Proximate costs of fighting in male cichlid fish: the role of injuries and energy metabolism. Anim Behav. 55:875-882.

Nilsson GE, Östlund-Nilsson S, Munday PL. 2010. Effects of elevated temperature on coral reef fishes: loss of hypoxia tolerance and inability to acclimate. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 156:389-393.

Otto RG. 1973. Temperature tolerance of the mosquitofish, *Gambmia affinis* (Baird and Girard). J Fish Biol. 5:575-585.

Parker GA. 1974. Assessment strategy and the evolution of fighting behaviour. J Theor Biol. 47:223-243.

Peden AE. 1972. The function of gonopodial parts and behavioral pattern during copulation by *Gambusia* (Poeciliidae). Can J Zool. 50:955-968.

720 Pyke G. 2005. A Review of the Biology of *Gambusia affinis* and *G. holbrooki*. Rev Fish Biol Fish.
721 15:339-365.
722 Pyke GH. 2008. Plague Minnow or Mosquito Fish? A Review of the Biology and Impacts of Introduced
723 *Gambusia* Species. Annual Review of Ecology, Evolution, and Systematics. 39:171-191.
724 Ratnieks FLW, Foster KR, Wenseleers T. 2006. Conflict resolution in insect societies Annu Rev
725 Entomol. 51:581-608.
726 Reddon AR, Balk D, Balshine S. 2013. Probing aggressive motivation during territorial contests in a
727 group-living cichlid fish. Behav Processes. 92:47-51.
728 Reddon AR, Voisin MR, Menon N, Marsh-Rollo SE, Wong MYL, Balshine S. 2011. Rules of engagement
729 for resource contests in a social fish. Anim Behav. 82:93-99.
730 Reeve HK. 1992. Queen activation of lazy workers in colonies of the eusocial naked mole-rat.
731 Sachs JL, Mueller UG, Wilcox TP, Bull JJ. 2004. The evolution of cooperation. Q Rev Biol. 79:135-160.
732 Seebacher F, Holmes S, Roosen NJ, Nouvian M, Wilson RS, Ward AJW. 2012. Capacity for thermal
733 acclimation differs between populations and phylogenetic lineages within a species. Funct
734 Ecol. 26:1418-1428.
735 Sinclair ELE, Ward AJW, Seebacher F. 2011. Aggression-induced fin damage modulates
736 trade-offs in burst and endurance swimming performance of mosquitofish. J Zool.
737 283:243-248.
738 Sloman KA, Taylor AC, Metcalfe NB, Gilmour KM. 2001. Effects of an environmental perturbation on
739 the social behaviour and physiological function of brown trout. Anim Behav. 61:325-333.
740 Sloman KA, Wilson L, Freel JA, Taylor AC, Metcalfe NB, Gilmour KM. 2002. The effects of increased
741 flow rates on linear dominance hierarchies and physiological function in brown trout, *Salmo*
742 *trutta*. Can J Zool. 80:1221-1227.
743 Smith CC, Sargent RC. 2006. Female fitness declines with increasing female density but not male
744 harassment in the western mosquitofish, *Gambusia affinis*. Anim Behav. 71:401-407.

745 Sokolowska E, Kulczykowska E. 2009. Environmental influence on maturation and dominance
746 relationships in the three-spined stickleback (*Gasterosteus aculeatus L*.): temperature
747 competes with photoperiod for primacy. Oceanol Hydrobiol Stud. 38:31-48.
748 Tatara CP, Mulvey M, Newman MC. 1999. Genetic and demographic responses of mosquitofish
749 (*Gambusia holbrooki*) populations exposed to mercury for multiple generations. Environ
750 Toxicol Chem. 18:2840-2845.
751 Tatara CP, Mulvey M, Newman MC. 2002. Genetic and demographic responses of mercury‐
752 exposed mosquitofish (*Gambusia holbrooki*) populations: Temporal stability and
753 reproductive components of fitness. Environ Toxicol Chem. 21:2191-2197.
754 Valderrábano-Ibarra C, Brumon I, Drummond H. 2007. Development of a linear dominance hierarchy
755 in nestling birds. Anim Behav. 74:1705-1714.
756 Ward AJ, Webster MM, Hart PJ. 2006. Intraspecific food competition in fishes. Fish Fish. 7:231-261.
757 Wasser SK, Barash DP. 1983. Reproductive suppression among female mammals: implications for
758 biomedicine and sexual selection theory. Q Rev Biol. 58:513.
759 Webster MS. 2004. Density dependence via intercohort competition in a coral-reef fish. Ecology.
760 85:986-994.
761 Whiteman EA, Côté IM. 2004. Dominance hierarchies in group-living cleaning gobies: causes and
762 foraging consequences. Anim Behav. 67:239-247.
763 Wiley RH, Rabenold KN. 1984. The evolution of cooperative breeding by delayed reciprocity and
764 queuing for favorable social positions. Evolution.609-621.
765 Wong M, Balshine S. 2011. Fight for your breeding right: hierarchy re-establishment predicts
766 aggression in a social queue. Biology Letters. 7:190-193.
767 Wong MYL, Buston PM, Munday PL, Jones GP. 2007. The threat of punishment enforces peaceful
768 cooperation and stabilizes queues in a coral-reef fish. Proc R Soc B-Biol Sci. 274:1093-1099.

769 Wong MYL, Munday PL, Buston PM, Jones GP. 2008. Fasting or feasting in a fish social hierarchy.
770 Curr Biol. 18:R372-R373.
771 Young AJ, Carlson AA, Monfort SL, Russell AF, Bennett NC, Clutton-Brock T. 2006. Stress and the
772 suppression of subordinate reproduction in cooperatively breeding meerkats. Proc Natl Acad
773 Sci. 103:12005-12010.
774 Young AJ, Monfort SL, Clutton-Brock TH. 2008. The causes of physiological suppression among
775 female meerkats: a role for subordinate restraint due to the threat of infanticide? Horm
776 Behav. 53:131-139.

FIGURE LEGENDS

Figure 1 Experimental design, showing the division of 20 acclimated mosquitofish groups (each consisting of five females and one male) into control and treatment conditions. Behavioural observations were conducted four times at monthly intervals.

Figure 2 Comparison of mean standard lengths at each dominance rank for both control and treatment groups across all observations. Shown are means ± SE.

Figure 3 Mean rate of aggression given towards adjacent ranked individuals and non-adjacent ranked individuals. Dark bars represent the aggression from subordinates to dominants and light bars represent aggression from dominants to subordinates. Shown are means ± SE.

Figure 4 Mean rate of aggression towards adjacent (circles) and non-adjacent (diamonds) ranked group members (N = 96) over four time periods. Shown are means± SE.

Figure 5 Mean rate of aggression towards adjacent (grey bars) and non-adjacent (hatched bars) individuals for each dominance rank (N = 96). Shown are means± SE.

Figure 6 Mean rate of aggression in control (circles) and treatment (diamonds) groups over four time periods (N = 96). Shown are means± SE.

Figure 7 Mean percentage growth rates of individuals in control circles) and treatment (diamonds) (N = 96) between observations 1-3. Zero represents no change in size over time. Shown are means ± SE.

Figure 8 Relationship between percentage growth rate of individuals (N = 76) (between observations 1-3) and the initial size ratio with respect to (a) the individual's immediate

dominant (DomSR) and (b) the individual's immediate subordinate (SubSR), for control (circles) and treatment (diamonds) groups. Lines represent linear regressions: (a) DomSR (Control: y = 0.080x - 0.012, Treatment: y = -0.30x + 0.30); (b) SubSR (Control: y = 0.25x - 0.18, Treatment: y = 0.19x - 0.17).

822 Table 1 Behavioural ethogram for *G. holbrooki*. Parenthesised letters indicate the short-
823 hand code used to score behaviours.

Behavioural category	*Behaviour*	*Description*
Aggressive	Chases (C)	Fish rapidly swims towards and follows a fleeing conspecific.
	Bites (B)	Fish bites another fish, usually on caudal fin. Often following a chase.
	Jolts/Thrust (J)	Rapid movement towards conspecific, but not sustained as in a chase.
	Aggressive posture (Ap_1 Ap_2)	Fish thrashes forming an "s-bend" display OR Fish circle each other, swimming parallel with stiffened bodies.
Submissive	Flee (F)	Fish rapidly swims away after a lost dominance encounter.
	Submissive posture (Sp)	Gentle side to side movement when confronted by a dominant.
Maintenance	Feed (Fe)	Focal fish orients towards food and opens its mouth to take in particles.
	Out of sight (OOS)	Fish cannot be seen either by entering the PVC shelter or swimming behind it.

Social Affiliation	Meeting (M)	Male and female simultaneously approach each other to within 1 body length.
	Follows (Fo)	Fish follows another fish within 1 body length for at least a few seconds.
Other	Darts (D)	Sudden quick swim in any direction, not directed at anything, not an obvious flee from a fish
Male Behaviours	Gonopodial swing (GS)	Gonopodium is moved away from its resting position underneath the body (Peden 1972; Krotzer 1990)
	Gonopodial thrust (GT)	Gonopodium is thrust towards the genital region of another individual (Peden 1972; Krotzer 1990)
	Copulation attempt (CA)	A male follows a female and makes repeated gonopodial thrusts towards female.

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